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Známky vzniku a evoluce Eusociality na genomické úrovni u blanokřídlých (Hymenoptera)

Signs of the Origin and Evolution of Eusociality in Hymenoptera on Genomic Level

Bakalářská práce

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Poděkování

Chtěla bych poděkovat mému školiteli za možnost věnovat se tak zajímavému a složitému tématu, kterým eusocialita je, za jeho milý přístup a rady, které mě vedly tímto rokem, svým rodičům za to, že jsou stále po mém boku a dodávají mi naději, svému příteli a kamarádům, jejichž optimismus je oponentem mého věčného pesimismu a hlavně všem učitelům, přednášejícím a profesorům, kteří mě provázeli a provázejí na cestě vědomostí a zápalu a bezmezně předávají znalosti nám všem, kteří po nich toužíme.

Prohlášení:

Prohlašuji, že jsem bakalářskou práci sepsala samostatně s použitím uvedené literatury a konzultací se školitelem. Práce nebyla předložena jako závěrečná práce k získání jiného nebo obdobného druhu vysokoškolské kvalifikace.

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Tereza Fraňková

Abstrakt

Eusocialita vznikla u blanokřídlých mnohokrát nezávisle na sobě, z nichž někteří zástupci blanokřídlých se právě teď nachází na sklonku eusociality. Eusocialita je komplikovaný set genomických, ekologických a behaviorálních vlastností, které spolu úzce interagují. Aktuální studie přinesly mnoho zajímavých výsledků, které alespoň částečně osvětlují možné vztahy eusociality a genomiky. Nicméně pravý původ a evoluce eusociality stále čeká na svoje odhalení. Objasnění příčin vzniku eusociality je u tak prominentního způsobu chování více než žádoucí. Tato teze shrnuje ty nejdůležitější objevy na poli genomiky a posuzuje nejen výsledky, ale i problémy tohoto odvětví, s kterými se vědečtí pracovníci setkávají při studiu tohoto fenoménu. Teze klasifikuje druhy eusociality, krátce pojednává o životním stylu druhů, díky čemuž lze lépe porozumět významu hlavní části této teze. Tato hlavní část se zaměřuje na konkrétní genomická data ukazující možné znaky eusociality, která nám jsou doposud známá.

Klíčová slova: eusocialita, genomika, evoluce, Hymenoptera, Insecta

Abstract

Eusociality has evolved independently many times in social Hymenoptera and some of them are now at the brink of eusociality. Eusociality is a complicated set of genomic, ecological and behavioural traits closely interacting with each other. Recent studies presented many interesting outcomes which explain at least partially the possible connections to eusociality on the genomic level. However, the true origin and evolution of eusociality is yet to be refined. Because eusociality is such a dominant quality in the lives of eusocial Hymenoptera, it is important to clarify what causes eusociality to arrise. This thesis summarises the most prominent findings in the field of genomics and reviews not only the outcomes but also the issues of this problematics. The thesis deals with the classification of eusociality, brief introduction to the species lifestyles, which are of great importance for understanding the main part. The main part focuses on the concrete genomic data elucidating possible signs of the evolution of eusociality recognised so far.

Key words: eusociality, genomics, evolution, Hymenoptera, Insecta

Obsah:

1. Introduction

Species of Hymenoptera are a part of our everyday lives. They have always fascinated us so much that we have even incorporated them even into cartoons, we have made entire movies about them. We use them as a way of communication in our everyday language, e.g. every time we hear that someone is "as busy as a bee" or that someone has "ants in the pants". They fascinated us so much that we have tried to decode the perfection of their social groups, how effectively they communicate and do their chores. The first one who mastered the Decoding the Language of the Bee was Karl von Frisch (1974). He analyzed what bees have on their minds while dancing in different ways but with the utter precision in their honeycombs, he explained what every movement means. However great a discovery this is, it was just the first step in understanding how their society works.

Since more advanced means of studying organisms such as molecular methods combined with bioinformatics, are now accessible to a wider range of people, they have opened up a whole new world to us.

Nonetheless, there is still one more question we now face and which baffles our minds even more: how did sociality arise in Hymenopterans and what are the common pathways or even specific genes that cause sociality to evolve? As much as we would like to know the answer it remains yet to be uncovered. Eusociality is considered to be the most advanced way of social organisation. The species conducting eusociality often sacrifice their life to the fellowship (Nowak et al. 2010). Darwin (1907) himself took interest in social groups of animals, where he mentions that any modification in the insects' social structure cannot be made without undisputable benefits for the whole community. The evolution of eusociality used to be explained by the 'kin selection theory', which considers the inclusive fitness as a main subject (Hamilton 1964). However kin selection theory does not consistently explain the basis of this matter and focusing on molecular or genomic studies is now, in the 21st century perhaps a way of correcting previous assumptions and not just closing the circle without further investigation. Many genomic studies have in the past come up with hints that lead us through the path while helping us find something that could give us a precise answer and these are the studies I am about to go through and review the points that could be benefitial to the progress in studying the matter of eusociality and even the points that do not answer the question, to offer a different point of view.

Since the Honeybee Genome Sequencing Consortium (2006) made the Honeybee genome available to groups focusing on eusociality and its origin, a better form of understanding of the aspects of eusociality has arisen. Still, onward research in the problematics of how eusociality was engineered into the genome of many of the Hymenoptera, especially focusing on the species on the brink of eusociality, is needed. As not many genomes of primitively social species are available, isolating the small details is even harder.

All these problems are an integral part of the research and are also important for the future progress in research therefore pointing them out is also an essential part of this text.

Classifying the degrees of sociality will firstly give us the general context of the organisation in the order of what might have been the sequence mirrored in the evolution and will further also delineate the general problems we encounter. In the next part, we will define major lineages and their ecological and behavioral traits which are important for classifying the changes in genomic structure. This will help us understand the significance of changes which are described in the main chapter.

The main chapter outlines the leading findings contributing to understanding the progress in studying the evolution of eusociality and points out not only the positive acquisitions but even the ones that helped disprove theories or concrete findings. This chapter summarised the drawbacks of the reviewed works and their conclusions.

2. Degrees of eusociality in Hymenoptera

Eusociality itself is defined by three traits. The first one is a cooperative brood care, where members of the family care for others, especially the young ones. The second one being that members of the colony should be separated into reproductive castes and non-reproductive castes and the third and the last point is, that their generations should overlap in order to get help from the offspring with rearing a next generation (Gadagkar 1994).

However, Crespi and Yanega (1995) define eusociality by only two of traits mentioned above, specifically the first and the second point, they do not mention the overlapping of generations which could be confusing. Wilson (1971) himself posits the three parametres for eusociality.

Despite the unstable definition of eusociality, eusociality itself cannot explain its origins because the workers of eusocial societies are different from their queen in terms of morphology, therefore they are behind the "point of no return" (Wilson and Hölldobler 2005). The traditional division of the degrees of sociality by Michener (2007) (Table 1) and Wilson (1971) will suffice for the purposes of this work (Crespi and Yanega 1995).

2.1. Solitary

Solitary species show none of the three aspects mentioned above (Wilson 1971). However, food providing for offspring is normal in solitary bees, the death of the female comes before the adult stage of life of her offspring. **Mass provisioners** also belong to the solitary term. These bees provision their offspring with a ball of pollent put in the cell of the nest. The cell is then closed (Michener 2007).

2.2. Communal

Communal species share a nest although tasks are not equally divided into the members of communal societies since no castes are present (Wcislo and Tierney 2009; Wilson 1971). Michener (1974) specifies the definition of communal behaviour to females who only take part in parenting of their own larvae but nest together. This degree also does not fulfill any of the three qualities of eusociality.

2.3. Quasisocial

One generation takes part in taking care of the brood and inhabits the same nest (Wilson 1971). Females cooperate in nest activities and use it for their own egg laying. Females are not relatives (Michener 2007).

2.4. Semisocial

Worker caste is present and takes part in caring for the brood of the reproductive caste (Wilson 1971). Females from one generation related or not even related can stay in the nest (Hogendoorn and Velthuis 1999). No overlapping generations are present.

2.5. Eusocial

Eusociality is defined by the reproductive division of labour, cooperation in brood care and overlapping generations (Michener 1974; Wilson 1971; Gadagkar 1994). Eusocial bees are **progressive feeders**, which means they feed larvae continuously, unlike mass provisioners (Michener 2000).

2.6. Subsocial

Subsociality is an attribute of sociality which can appear in either degree of sociality. Interactions between brood and parents appear, they, however, do not fulfill any of the three qualities of eusociality (Tallamy and Wood 1986). Parents care for their brood for a period of a time (Wilson 1971) not only preparing food for the larvae but actively feeding or caring in other ways such as grooming (Michener 2007).

2.7. Primitively eusocial colonies

Solitary females found a colony and later becomes a progressive feeder. Colonial lifestyle uprises with the emergence of the first generation of daughters which are not morphologically different from the founding female. They can be slightly smaller and physiologically and behaviourally different (Michener 2007).

	Qualities of sociality		
Degrees of sociality	Cooperative brood care	Reproductive castes	Overlap between generations
Solitary, subsocial, communal	-	-	-
Quasisocial	+	-	-
Semisocial	+	+	-
Eusocial	+	+	+

Table 1. Basic division of sociality by Wilson (1971).

3. Species lifestyles

In this thesis, various species from different lineages are introduced. This chapter will contain information about the social lifestyle of the species that can provide a general understanding of their social complexity (Figure 1) and ecology and therefore understanding of the data presented in this thesis. The phylogenetic study of Hymenoptera from Peters et al. (2017) will be utilized for the purposes of this paper. Other publications will be used as a more specific classification in terms with Peters et al. (2017).

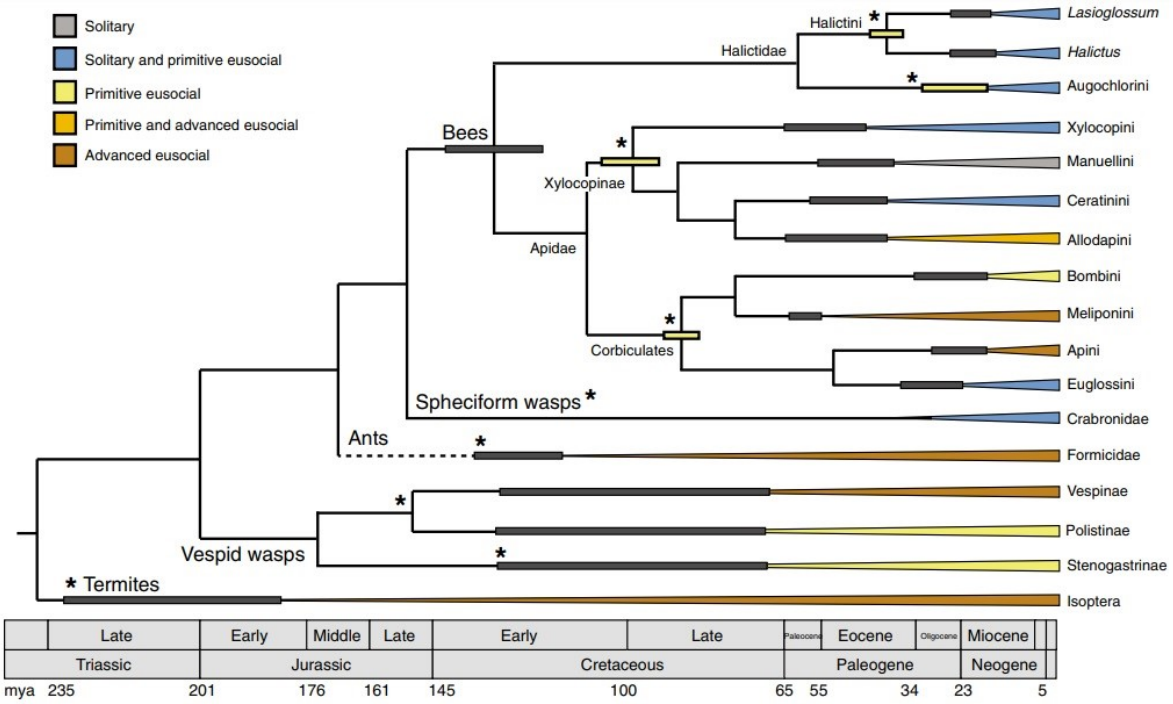

Figure 1. Simplified phylogenetic tree of Hymenoptera with accented degrees of eusociality. This phylogenetic tree shows tentative divergence times of Vespid wasps, Ants, Spheciform wasps and Bees together with an advanced eusocial taxa Termites as an outgroup. Distinct types of eusociality are differentiated by coloures captioned in the left upper corner. (Kocher and Paxton 2014).

3.1. Vespidae

3.1.1. Polistinae

Polistinae comprises of advanced subsocial to eusocial species (Wilson 1971) whose life cycle is differentiated into three phases. First, the founding phase is usually initiated by one or several foundresses. Females can also wait until a nest is abandoned and take over the colony. First generation is a generation of workers, this is the time when the life cycle transfers into the worker phase. The dominant female is the only one allowed to oviposit and initiate new cells. The dominant female performs queen policing, where eggs laid by workers are eaten by the dominant female (this activity is called oophagy). Some dominant workers can replace the dominant female (referred to as a queen). The second generation reared with the help of workers is going to be a generation of gynes (the third phase),

which will later become reproductives. When the dominance is settled, tasks in the nest are divided. Since hybernation of reproductives is common, Polistinae have an annual life cycle (Jandt et al. 2014). The queen is often larger than anybody else in the nest (West-Eberhard 1969). Single mating is a standard for Polistinae (Strassmann 2001). Polistinae are also progressive provisioners (Hines et al. 2007).

- *Polistes canadensis*

  Here only one female initiates a nest and establishes her dominance by aggressive behaviour towards other foundresses. An active queen must be present in order for the normal colony function - which consists of nest expansion and brood care. When a queen is not present, new queen can substitute the first one and the nest continues, otherwise it deceases (West-Eberhard, 1962).

- *Polistes dominulus*

  The life cycle of *Polistes dominulus* is comparable to *Polistes canadensis* (West-Eberhard 1962).

- *Polistes metricus*

  Polistes metricus is of two types, either a colony started by a single foundress or by multiple foundresses. Colonies with many foundresses are more successful in rearing the first worker generation early. There is always one worker in the first generation that will become a foundress (Gamboa 1980). Also, workers rather cooperate with former nest mates in new nests than with totally unrelated individuals (Ross and Gamboa 1981).

3.1.2. Vespinae

- *Vespula vulgaris*

  Vespula vulgaris is a eusocial species (Sirviö et al. 2011). Cavities in tree trunks and soil are the target for nest building (Donovan 1984). The queen is the only individual that hybernates. In the spring, the queen chooses the nesting site and constructs cells of the nest out of the wooden pulp she makes from chewing pieces of dead wood and vegetables. The queen lays eggs and when the first brood emerges, she provides them with catched insects which are chewed into a pulp and fed to the brood. The adult brood forages later on its own, the queen does not participate in any other activities than laying eggs. The adult brood foragers take over the building of the nest (Wilson 1971). Queens and workers are differentiated by their morphological traits. The life cycle is annual and only a single queen is present in the nest (Foster and Ratnieks 2001). Worker policing occures in *Vespula vulgaris* because of multiple mating of the queen (Foster and Ratnieks 2001).

3.2. Formicidae

All Formicidae are a highly eusocial species (Grimaldi and Agosti 2000).

3.2.1. Ponerinae

- *Dinoponera quadriceps*

  In ponerine, there is no queen caste but workers take part in laying eggs. These workers are called gamergates and there is only one dominant gamergate per colony (Peeters and Crewe 1984; Monnin and Peeters 1998). Societies of *Dinoponera quadriceps* are monogynous and mating happens only once in the beginning of the gammergate transition (Monnin and Peeters 1998). Dominance of the gammergate, also called alpha, is established by aggressivity and by consuming eggs of the opponent or "gamergate" quarding (which is similar to the queen guarding). This alpha lays most of the eggs (Heinze et al. 1994; Monnin and Peeters 1998). The gamergates spend time caring about the eggs. Nest defending and foraging are uncommon for them, these tasks are taken over by the lower ranked workers (Monnin and Peeters 1999).

- *Herpegnathos saltator*

  The colony is established by a queen which is chosen by an agreement among other members of the colony. When the queen dies, gamergates replace her in her position. The queen provides food to her offspring by hunting, when the first brood adults emerge, workers (gamergates) of this brood replace the queen in brood care, nest supervising and food providing (Peeters et al. 2000).

3.2.2. Myrmicinae

- *Pogonomyrmex barbatus*, *Pogonomyrmex rugosus*

  Multiple mating occurs in *Pogonomyrmex* species, life cycles are perennial (Hölldobler 1976). After mating, only one mated queen from the reproductive caste that left her parental colony founds a new colony. This queen is not replaced after her death and colony dies with her. The colony can live up to dozens of years (Gordon and Kulig 1996). Queen mates with multiple males (Suni et al. 2007).

- *Solenopsis invicta*

  Queens undergo only one mating with one male (Ross 1993). Queens can found a colony alone or with other newly mated queens, therefore a colony can have one or multiple queens (Tschinkel and Howard 1983). Societies of *S. invicta* consist of two type worker caste - nurses and foragers. Queens differ morphologically from worker caste (Mirenda and Vinson 1981). Colonies which consist of multiple queens produce worker castes equally in similar numbers (Ross 1988). Colonies are perennial (Tschinkel and Howard 1983) and worker policing occurs - workers can eradicate a queen that is the least productive (Gobin et al. 1999).

- *Acromyrmex echinatior*

  Colonies of *A. echinatior* can be facultatively polygynous. Multiple mating occures in queens (Bekkevold et al. 1999). Castes are morphologically differentiated (Hughes et al. 2003) and unlike queen policing, which does not occur, worker policing may occur in *Acromyrmex* colonies since young workers are capable of laying eggs of the same size as the queen eggs (Dijkstra et al. 2005).

- *Atta cephalotes*

  Atta cephalotes is a species of interest partially because of its fungus related behaviour (Hodgson 1955). It is a common eusocial species where multiple castes are present (Quinlan and Cherrett 1979). Queen oophagy might occur in her presence, young workers produce inviable eggs (Dijkstra et al. 2005).

3.2.3. Formicinae

- *Camponotus floridanus*

  Queens are mated by only one male (Gadau et al. 2008). Worker policing probably does not occur according to the new study but was recognised in the previous studies (Endler et al. 2007; Endler et al. 2004) queen policing is present (Endler et al. 2004). Castes are morphologically divergent (Zube and Rössler 2008).

3.2.4. Dolichoderinae

- *Linepithema humile*

  Linepithema humile societies have multiple queens in their colony (polygyny). The queens are singly mated (Krieger et al. 2001). Number of queens correlate with number of workers and the structure of the colony is highly influenced by ecological factors (Ingram 2002).

3.3. Halictidae

In Halictidae, eusociality emerged twice (Gibbs et al. 2012).

3.3.1. Halictinae

Halictinae are mass provisioners (Wilson 1971; Michener 2007) which built nests in the soil or wood. All nests look similar either solitary or primitively social (Michener 1969). Mated females overwinter and found nests in the spring which are either solitary, communal or semisocial. In semisocial societies, some females become workers and die unmated, males are produced in late summer, mate and then die. Usually, castes do not differ morphologically, but in size in some species.

- *Lasioglossum albipes*

  Lasioglossum albipes is a socially polymorphic species which has a eusocial and a solitary form. They produce one generation per year. In the spring, a nest is founded and the first brood is produced. This first brood helps with the second brood, which consists of reproductives (females and males). After mating, diapause is a part of their life cycle. The whole life cycle is repeated

the next year (Plateaux and Packer 2000). For another *Lasioglossum* species there was no evidence found which would support the multiple mating of the founding female (Davison and Field 2018).

- *Lasioglossum malachurum*

  Lasioglossum malachurum is very similar to *L. albipes*. Founding female of *L. malachurum* can mate with multiple males (Paxton et al. 2002). Queen policing is not present in *L.malachurum* (Richards et al. 2005)

- *Halictus rubicundus*

  Halictus is a socially polymorphic species of solitary lifestyle in higher altitude regions and eusocial at lower altitude regions (Eickwort et al. 1996). In its eusocial form of lifestyle, two types of female castes are produced, non-gynes and gynes. Gynes abandon the society because of overwintering. Yanega (1989) suggests that an overwintering gyne can be any female that mates sooner than the others. Nests are founded by single females, multiple mating is unlikely (Douglas Yanega 1990). Cells with larvae are temporarily opened in *H. rubicundus* (Michener 1969).

- *Megalopta genalis*

  M. genalis is a facultatively eusocial species where solitary and social females occur (Wcislo et al. 2004; Michener 2007). The solitary females tend to be smaller in their body size and they do not reproduce as soon as eusocial females. Also, reproductional females produce the first brood as a worker brood that helps them raise reproductives in the next brood (Kapheim et al. 2013). Only a single female founds a nest. The dominant females are bigger than subordinates (Wcislo a Gonzalez 2006).

3.3.2. Rophitinae

- *Dufourea novaeangliae*

  Dufourea are solitary species. When foraging, cells with brood are opened. At the end of a day cells are sealed and the nest protected. A female provides an offspring with a pollen ball on top of which she lays the egg (Eickwort 1985).

3.4. Apidae

3.4.1. Apinae

- *Apis mellifera*

  Apis mellifera is a prototype of eusocial species. All of the three qualities of sociality are present and their workers cannot become queens (Wilson 1971). Castes are morphologically very different (Michener 2007), multiple mating is present (Page 1980) which is together with the mere presence of the queen a cause for worker policing in *A. mellifera*. Swarming is an activity when the old queen flies to find a new nest with a part of her daughter workers in order to found

a new nest which is then established. The old colony acquires a new queen which has been fed into her status by the major royal jelly. The first virgin queen that emerges from the cells can fight with the next virgin queen that emerges from the next cell. If she wins she can then participate in nuptual flights where she is multiply mated. Colonies of *A. mellifera* are perennial (Wilson 1971)

- *Apis florea*

  Apis florea, sometimes called a dwarf honeybee, builds opened single-combs. Queens also mate with multiple males as in *Apis mellifera* (Oldroyd et al. 1994) and worker policing can occur between workers together with oophagy of worker eggs (Ratnieks and Visscher 1989; Halling et al. 2001). Reproduction and swarming is similar to that of *A. mellifera*, nests and colonies are perennial (Free 1981).

- *Bombus terrestris*

  Bombus are primitively social species and progressive provisioners (Michener 2007) where queens are singly mated and found annual colonies. Multiple generations occur during the season, sons and daughters that leave the nest and mate are produced in the last generation. Mated females then found a new colony, each of the colonies have only one queen (Schmid-Hempel and Schmid-Hempel 2000). Workers are smaller but morphology is the same for workers and queens (Michener 2007).

- *Bombus impatiens*

  The life cycle of *B.impatiens* is very similar to the life cycle of *B. terrestris* (Schmid-Hempel and Schmid-Hempel 2000; Michener 2007).

- *Habropoda laboriosa*

  Habropoda laboriosa is a representative of solitary bees. The annual nests are excavated in the soil. The bee provides cells in the nest with nutrition (Cane 1994), which makes it a mass provisioner (Michener 2007).

- *Melipona quadrifasciata*

  Meliponini are mass provisioners that live in highly eusocial societies. Queen and worker castes are morphologically and behaviourally different. A new nest is built in the ground by workers from the old nest. A new young queen moves to a newly built nest and workers that prepared the nest stay with the new young queen. Worker policing is not developed, because Meliponini are usually singly mated (Michener 2007).

- *Eufriesea mexicana*

  Eufriesea are a solitary species whose nests are founded in cavities where eggs are laid in the cells and mass-provisioned. Single mated female founds the colony (Michener 2007).

3.4.2. Xylocopinae

- *Ceratina calcarata*

  Ceratina calcarata is a solitary subsocial species whose nests are located in twigs. Mothers founding nests are taking care of their offspring until their adult stage of life. *Ceratina* is a mass provisioner (Michener 2007) which lives a long life and is loyal to its nest (Rehan and Richards 2010).

- *Ceratina australensis*

  Founding females do not mate with multiple males, social biology is similar to *Ceratina calcarata*. However, *C. australensis* is facultatively eusocial (Rehan et al. 2014a, 2014b).

3.5. Megachilidae

3.5.1. Megachilinae

- *Megachile rotundata*

  Megachile rotundata is a solitary bee species (Blanchetot 1992). In Megachilinae, nests are built in various cavities from soil to snail shells (Michener 2007). Brood is provisioned with nutrition in the form of a pollen ball (Klostermeyer and Gerber 1969)

3.6. Taxa used as outgroups

- Drosophila melanogaster (Diptera)
- Aedes (Diptera)
- Nasonia vitripennis (Hymenoptera)
- Manduca sexta (Lepidoptera)
- Bombyx mori (Lepidoptera)
- Tribolium (Coleoptera)
- Acyrthosiphon pisum (Hemiptera)

4. Juvenile hormone (JH) and Vitellogenin

Juvenile hormone is a very important mechanism of caste differentiation and of maintaining a juvenile characterisation in the life of an insect (Williams 1956). Juvenile hormone is not directly connected to genomics, however, the importance of Juvenile hormone for Hymenoptera and the indirect effect on the genome is not negligible. Titres of JH in *A. mellifera* during the larvae ontology of the queen and worker differ, JH in the queen is usually higher than in the worker. However queens body is much bigger than that of a worker, the titer amount of JH in the queen could be the reflection of her body size (Rembold 1987). Juvenile hormone titres are also connected to the switch from nursing behaviour to foraging behaviour in *Apis mellifera*. A bigger amount of JH titre and a smaller amount of vitellogenin is connected to the switch from nursing to foraging (Guidugli et al. 2005). Ihle et al. (2010) performed a

knockout on vitellogenin mRNA in *A. mellifera*. Workers with the knockout were pushed to forage earlier than the other workers which were not a target of vitellogenin knockout and their life cycle is marked by a natural vitellogenin production. This finding of the relationship between vitellogenin and JH in the release of foraging behaviour is consistent with the work of Guidugli et al. (2005) and also according to Ihle et al. (2010) this relationship resulting in foraging is called the double repressor hypothesis (DRH) (Figure 2), which is a "positive control between the expression of JH and the allatoregulatory central nervous system". The allatoregulatory central nervous system is under negative regulatory control by an internal repressor (haemolymph titre of the lipoprotein vitellogenin) and an external repressor (physiological materialization inside the bee hive of a signal originating from foragers which is transfered by physical contact). The loss of external repressor induces transition from hive to forager bee by activating the allatoregulatory central nervous system pathway. This induces the JH-independent differentiation pathway and increase of the JH production. Higher levels of JH production act as an inhibitor to the vitellogenin synthesis. This activates a JH-dependent differentiation pathway. Repression of the synthesis of vitellogenin induces self-reinforcing positive feedback loop which maintaines the activity of the allatoregulatory central nervous system pathway. Aftewards the bee stays in the forager stage on both the behavioural and physiological level (Amdam and Omholt 2003). The opposite of a double repressor hypothesis is the single-function hypothesis that stands for a shift in the function of JH from an exclusively reproductive function in primitively eusocial species to an exclusively behavioral function in highly eusocial societies. The split-function hypothesis (which proposes that both functions, regulation of behaviour and reproduction, were administered by JH) participated in ancestral solitary species before the reproductive and brood-care tasks were divided between workers and queens (Giray et al 2005). In a paper wasp *P. canadensis*, titres of JH were highest in foundreses – the reproductives. However, JH titres in workers, which were low, did not correlate with ovary development or mating status, JH titres in egg-laying queens were highly correlated with ovarian development. These findings contradict the single-function hypothesis (Giray et al 2005). Tibbets and Izzo (2009) measured phenotypic plasticity in another paper wasp *P. dominulus* via JH analog – methoprene which increased the number of mature eggs in an individual's ovaries. The results were not homologous across all individuals.

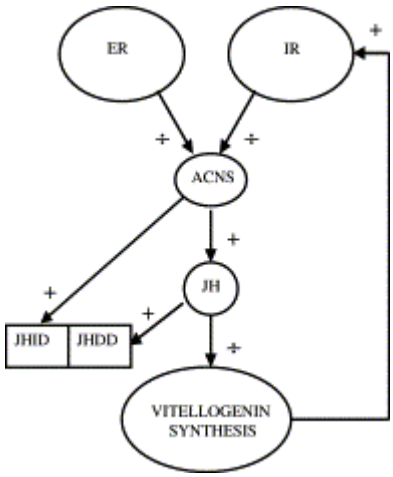

Figure 2. The double repressor hypothesis scheme. IR (internal represor), ER (external represor), ACNS (allatoregulatory central nervous system), JHID (Juvenile hormone-independent differentiation), JHDD (juvenile hormone-dependent differentiation), JH (Juvenile hormone) (Amdam and Omholt 2003)

In moth *M. sexta*, which can be used as an outgroup, reduction of feeding was an impulse for the reduction of JH levels (Lee and Horodyski 2002). In bees there is a similarity in nutrition and JH signaling that determine the caste fate of an individual. However, the connection between factors is still unclear (Mutti et al. 2011). In young queenless workers of *B. terrestris* JH synthesis and ovarian

development is prominent. The queenright workers of similar age did not synthesise JH to such extent. Dominant workers in queenright colonies had a higher rate of JH biosynthesis and ovarian development. Lower ranked workers of similar size had a lower rate of JH biosynthesis and ovarian development. These results provided by Bloch et al. (2000) suggest that JH is the main factor influencing the reproduction in *B. terrestris*.

Unlike in an ant *H. saltator*, JH which was given to virgin queens, did not induce egg production at all. Juvenile hormone titres were higher in foragers (which is a similar outcome to that of a honeybee) and did not differ among workers, gamergates or queens. The juvenile hormone appears to have lost the egg production function in *H. saltator* (Penick and Brent 2011).

In ant *P. rugosus* the main mechanism which initiates the vitellogenin production is hybernation and the JH, which was examined by Libbrecht et al. (2013). During hibernation, JH titres increase therefore vitellogenin synthesis is induced. Also, the relationship between vitellogenin and JH in *P. rugosus* helps produce different phenotypes of individuals and has several maternal effects. Vitellogenin in a related ant *P. barbatus*, on the other hand, expresses 2 Vitellogenin genes (Pb_Vg1 and Pb_Vg2) where Pb_Vg1 is more expressed in queens and nurses and Pb_Vg2 is more expressed in foragers. This is a result of duplication of the Vitellogenin gene and differentiation from its ancestral function towards caste and behavioral specific function (Corona et al. 2013). *Solenopsis invicta* concludes more than 30 putative JH binding protein encoding genes. Vitellogenin is expressed in workers (which is unexpected because workers do not have active ovaries) and queens. Vitellogenin in workers probably gained a new function (Wurm et al. 2011). Dominant females in another ant, *P. metricus,* have higher levels of JH than subordinates. Vitellogenin could have maintained its primal function (Toth et al. 2009).

5. Signs of the Origin and Evolution of Eusociality

5.1. Recombination

Differences in genome are needed for higher fitness of colonies. The fitness is reflected in overwintering with overall surviving numbers and drone production therefore recombination reacts to natural selection (Mattila and Seeley 2007; Sirviö et al. 2011). Polyandry (where female mates with multiple males (Arnqvist and Nilsson 2000)) and recombination is connected to genetic diversity in social hymenoptera (Oldroyd and Fewell 2007). Eusociality itself enhances recombination (Ross et al. 2015), which together with polyandry helps protecting highly genetically diverse populations from parasites (Hughes and Boomsma 2004; Baer and Schmid-Hempel 1999).

An ant, *Pogonomyrmex rugosus*, has the recombination rate of 13.9 cM/Mb (Sirviö et al. 2006) which is comparable to *A. mellifera* with the rate of 16 cM/Mb (Wilfert et al. 2007). Another ant, *Acromyrmex echinatior*, has the recombination rate of 6.2 cM/Mb (Sirviö et al. 2006) which is significantly lower than the recombination rate of *A. mellifera, Acromyrmex* is a eusocial species as well. Still, those numbers are much higher than those of a parazitoid wasp *N. vitripennis*. Recombination for eusocial

taxa are higher overall than for solitary or parasitoid taxa (Table 2) such as *N.vitripennis*, where the recombination rate is 1.4-1.5 cM/Mb (Niehuis et al. 2010). *Vespula vulgaris*, another social species, has the recombination rate also high, at 9.7 cM/Mb (Sirviö et al. 2011) although not as high as in *A. mellifera*. *Bombus terrestris* in Gadau et al. (2001) was thought to have the recombination rate of 3.91 cM/Mb (Gadau et al. 2001), however, new computations refined the results to 4.4 cM/Mb (Wilfert et al. 2006). Similarly, *A. florea* has a similar recombination rate to *A. mellifera,* however, only 12.9% of the physical genome map and 10.8% of the recombinational map (Meznar et al. 2010) were used in this study so these outcomes might be uncertain and *A. florea* will not be included in the Table 2. Further research should be conducted in order to clarify this matter, nonetheless existing outcomes are still of value for this research.

In *C. calcarata*, genes associated with DNA recombination (Ccalc.v2_001846, , Ccalc.v2_012690, Ccalc.v2_004327, Ccalc.v2_013055) have undergone a faster evolution (Rehan et al. 2016). This might indicate the transfer to a more complex social lifestyle and hence higher DNA recombination, even though we do not have the precise recombination rate for *C. calcarata* which would confirm the suggestion.

	Species	Haploid chromosome number	Linkage size of genome (cM)	Physical size of genome (Mb)	Recombination rate (cM/Mb)	Source:
Bees	*Apis mellifera*	16	4192	262	16	(The Honeybee Genome Sequencing Consortium 2006)
	Bombus terrestris	18	2760	625	4.4	(Wilfert, Gadau and Schmid-Hempel 2006)
	Ceratina calcarata	unknown				(Rehan et al. 2016)
Ants	*Pogonomyrmex rugosus*	Not yet published	3558	225	15.8	(Sirviö et al. 2006)
	Acromyrmex echinatior	18	2076	334.8	6.2	(Sirviö et al. 2006)
Wasps	*Vespula vulgaris*	25	2129	219.8	9.7	(Sirviö et al. 2011; Hoshiba, Matsuura and Imai 1989)
	Nasonia vitripennis	5	446	312	1.4-1.5	(Niehuis et al. 2010)

Table 2. Summary table for haploid number of chromosomes, linkage size of genome, physical size of genome and recombination rate of several representatives.

5.2. Methylation, acetylation and CpG content

Methylation is an epigenetic mechanisms which serves as a modifier of gene regulation and therefore, phenotypic plasticity. Examining this mechanism, which is widespread across social Hymenoptera (Kronforst et al. 2008), is a promising means for indicating evolutionary shifts in eusociality. The presence of DNA methylation proteins DNMT1, DNMT2 and DNMT3 – DNA methyl transferases, is requiered in order to perform a functional genom-wide methylation (Yi and Goodisman 2009). Methylation can be reduced to almost a zero in organisms (Finnegan et al. 1996; Standage et al. 2016). DNA methylation in *L. albipes*, according to Kocher et al. (2013) may play a role in a social behaviour regulation. DNA methylation system of *L. albipes* consists of the full DNMT kit. Methylation is an active process in *L. albipes* due to the strong support of CpG O/E (Observed/Expected) ratios displaying clear and consequential characteristics from the genomic background examined (Kocher et al. 2013). To understand why CpG O/E ratios are important, Yi and Goodisman (2009) explain that cytosine followed by guanines loci (CpG dinucleotides 5' → 3') are the target of DNA methylation. Almost entirely, methylation occures at this loci (Yi and Goodisman 2009). Kocher analysed candidate genes for methylation, which needs further investigation in comparative genomics and transcriptomics both interspecificly and intraspecificly in terms of different degrees of sociality.

A few differences between the methylation of genomes in queenless reproductive workers and queenless non-reproductive workers in bumble-bee *B. terrestris* were found (Amarasinghe et al. 2014). Invoking results, a new comparative study of two bumblebees *B. impatiens* and *B. terrestris* examined methylation in these species. Here, DNA methylation maintains describing features similar to those of *A. mellifera,* which imprints an equally important role in conducting caste differentiation and behavioral divergence in species with primitive eusocial organisation as in eusocial species behind "the point of no return" (Sadd et al. 2015). It may also be closely related to the development of ovary and reproduction of the queen in bumblebee *B. terrestris* (Li et al. 2018).

Genes enriched for Gene Ontology (GO) biological processes in *C. calcarata* had a part in core cellular functions and translation. Those enriched genes were methylated and their expression was found in RNA seq data, says Rehan (2016). In the preceding study, Kapheim et al. (2015) mentions that the species with more complex form of eusociality are more targeted by gene regulation. Gene regulation is therefore more expressed in eusocial species than in primitively social species. However, methylation in *A. mellifera* is smaller (6.2% ortholog pairs with significant DNA methylation) than in facultatively eusocial *C. calcarata* (11.9%) and this result is a contradiction to the prediction (Rehan et al. 2016). This discovery further supports Glastad et al. (2017), who recently performed a study comparing one solitary, one facultatively eusocial halictid bee and a sawfly. According to their results, DNA methylation might not be a representative mechanism connected to the evolution of eusociality. There is also no evidence for DNA methylation to be consistent across species.

Comparing a wasp *P. canadensis* and an ant *D. quadriceps*, both living in simple eusocial societies, Patalano et al. (2015) did not detect any confirmation that DNA methylation, not even miRNA induces

phenotype-specific gene expression. According to Patalano et al. (2015), phenotype-specific gene expression is defined more subtly by nonrandom transcriptional network organisation which affects conserved, even taxon-restricted genes. Modifications in both species do not incorporate DNA methylation therefore the absence of methylated regions could be a dominant factor which modifies the plasticity in phenotypes mainly in the adult phase of their development according to Patalano et al. (2015).

The genome of an Argentine ant *L. humile* suggest an active methylation. The highest CG → TG bias (sign of a methylation) in genome is expressed in Major Facilitator Superfamily Transporters, which consists of male sterility proteins, several classes of Zinc Finger Transcription Factors and several Ig Superfamily Cell Adhesion proteins entangling in neuronal development. More prominent methylation can also be found based on the CpG O/E ratios in genes connected to apoptosis and reproductive development (Smith et al. 2011).

Another ant, *P. barbatus* has figures of CpG O/E in exon similar to insects without CpG methylation (Glastad et al. 2011) thus suggesting low frequency of germline methylation. However, methylation is enhanced in the main differenceal atributes while defining caste of queen and worker, such as ovaries and wings. Also a role of DNA methylation in genome regulation for which Smith et al. (2011) used Gene Ontology (GO) analysis, detected noteworthy enrichments in genes associated with recognition, sensory perception of smell, and neurological processes. A paper wasp *P. dominula* does not have a full methylation kit. The DNA methyltransferase 3 which stands for the de novo methylation is missing. Methylation almost does not occur in these species (Standage et al. 2016) therefore the hypothesis of positive corelation between eusociality and methylation does not generally apply.

Solenopsis invicta has a full methylation-kit, identified methylated genes were enriched for biological processes linked to cellular metabolism and transcription (Wurm et al. 2011) the same for *A. mellifera*, where low CpG content (the sign of methylation) is enriched for cellular metabolic processes (GO:0044237), RNA processing (GO:0006396) (Elango et al. 2009).

5.3. Expanded/contracted gene families

5.3.1. Chemoreceptors

Wilson (1971) affirmed the thesis that pheromones play a central role in the organisation of insect societies. Notwithstanding the truth, there should be an advance in chemical communication not only in the organisation of insect societies, but enhanced in more complex societies and in comparison, reduced in primitively social species due to the demanding challenges of transition into eusociality.

According to Zhou et al. (2015), evolution of chemoreceptors is a hypothesized but considerable part of the evolution of eusociality. To evaluate the contribution of chemoreceptor genes to eusociality, Yan et al. (2017) performed a knockout of orco gene in *H. saltator*. This gene encodes the obligate co-receptor, which mutation should impact ant olfaction. Using CRISPR-Cas9 a germline mutation in the orco gene

was generated. Thanks to the knockout, orco gene exhibited a conserved role in reproductive physiology, in the perception of general odorants and social behavior plasticity in ants.

Ceratina calcarata genome exposed 16 members of odorant receptors gene family which according to Rehan et al. (2016) is analogous to the gene family expansion exhibited in other eusocial lineages in the family. Therefore in a facultatively eusocial apid bee is presumably not directly connected to eusociality. Two prominent articles, investigating the genom of halictid bees *L. albipes* (Kocher et al. 2013) and *M. genalis* (Jones et al. 2015), do not take chemical communication and chemoreceptors into account at all. Studying socially polymorphic (*L. albipes*) or facultatively eusocial (*M. genalis*) bees from this perspective could give us some traces of common paths leading to eusociality in terms of communication.

Comparing genomes of ants *C. floridianus* and *H. saltator*, both socially and ecologically divergent species, Bonasio et al. (2010) found 506 enrichments of ant-specific genes in GO terms considering olfactory receptor activity, sensory perception of smell, odorant binding and others. In this study, homology-based approach looking for homologues of *D. melanogaster* was used therefore ant-specific olfactory receptors (ORs) were not detected. Expression level differences were detected in both, *C. floridanus* and *H. saltator* concerning chemical comunication and neuronal function.

A wide phylogenetic analysis of the ORs and gustatory receptors (GRs) genes in 13 solitary and social hymenopterans was used to find out when gains and losses of OR genes and GR genes might have happened. For the suitable ancestors of Apocrita, Aculeata, the three solitary wasps and the two bees, a small number of chemoreceptor genes were found. Zhou et al. (2015) suggests that many chemoreceptor genes found in wasps and bees are mainly the outcome of lineage-specific gene expansions. In ants, some events of duplication and loss events were detected. These events happen in high rates despite having a similar amount of ORs.

As *A. mellifera* genome reports 160-170 ORs (which is approximately the number of glomerulli in the bee antennal lobe), *N. vitripennis*, a parasitoid jewel wasp, on the other hand has 225 ORs (Robertson et al. 2010). *Nasonia* is a nonsocial species (Robertson and Wanner 2006) therefore chemoreceptors might be influential to the evolution of eusociality but their number does not directly correlate with the level of social organisation. Olfactory receptors are highly connected not only to the demands of social life but also to the ecology of species.

Roux et al. (2014) performed an analysis of seven ant genomes. The positive selection of amino-acid substitutions was surprisingly less frequent in ant than in wasp branches (using *N. vitripennis* as an outgroup from Hymenoptera), which also challenges the hypothesis of more complex olfactory functions due to more complex social organisation. In *P. barbatus*, the signs of expansion of ORs family is intense, 399 genes in total, the largest total known for any insect, is in comparison with *A mellifera* and *N. vitripennis* above noticable (Smith et al. 2011). This result was topped by the study of *Solenopsis invicta* which found more than 400 loci for ORs (Wurm et al. 2011). *Linepithema humile* has an expansion of odorant receptor genes to 367 genes, which is similar to *P. barbatus* (Smith et al. 2011).

Garnering these mixed signals getting from studies might be the reason for the low chances of complex comparative genomic research, also due to the lack of annotated genomes available and a wide variety of behavioral and ecological ranges of species.

5.3.2. Yellow and Major Royal Jelly proteins with connection to Juvenile hormone

Yellow genes and major royal jelly proteins have functions with various roles ranging from physiology to development. Many genes from these families act as coordinators of reproductive development (Drapeau et al. 2006). Major Royal Jelly Proteins (MRJPs) are very important for the caste differentiation and physiology of nurses (Johnson and Linksvayer 2010) since MRJP's expression is located in hypopharingeal glands (Santos et al. 2005).

A single progenitor gene was a connection to newly evolved MRJP protein family. Yellow protein family, which is also encoded by this progenitor gene (The Honeybee Genome Sequencing Consortium 2006), derived from *D. melanogaster* where the Yellow gene functions in the pigmentation of the cuticule. Major Royal Jelly Proteins have obtained a new function in *A. mellifera* (Albert et al. 1999). In *L. albipes*, 10 Yellow genes were found (the same number as in *A. mellifera*) however only two of the ten MRJP genes in *L. albipes* are plausible (in contrast with all 10 in *A. mellifera* (Fischman et al. 2011)) (Kocher et al. 2013). A similar number of MRJP genes can be found in ants *C. floridanus*, which have one, and *H. saltator*, which have 2 MRJP paralogues (Bonasio et al. 2010). Kapheim et al. (2015) compared 10 bee species with differences in social complexity and found only one expansion of MRJP family in *A. mellifera,* which is in agreement with Kocher et al. (2013) findings in *L. albipes*.

The largest number of Yellow/MRJP genes found in any insect, says Werren et al. (2010), is present in *N. vitripennis*. However, the functions of these genes in *N. vitripennis* are not clarified. Bonasio et al. (2010) compared this parazitoid wasp *N. vitripennis* with honeybee *A. mellifera* and thinks that ants might have lost their MRJPs in the evolution, or that the occurrence of MRJPs is independent in both, wasp and bee lineages. Also the hypothesis that adjustments of MRJPs functions and duplication correlate with eusocial complexity is probably wrong since *N. vitripennis* and ant *C. floridianus* have enormously different lifestyles and different amount of MRJPs. According to Kupke et al. (2012) the fact that *N. vitripennis* has the most MRJPs is a challenge to the view of so called uniqueness of advanced eusocial species. Also, it is very difficult to find the prime function of the MRJP genes in this time. Kupke et al. presented BtRJPL (Bombus terestris Royal Jelly Protein Line) found in *B. terrestris* which shares some similarities with honey bee MRJPs. These similarities are genome organisation, origin and expression pattern. Its function is related to food digestion or modification but it is not connected to the function of a nutritive type as in the honey bee. In ant *L. humile*, 10 *yellow* genes and 10 MRJP-like genes have been detected, MRJP-like genes are similar in numbers to *A. mellifera* and *N. vitripennis* (Smith et al. 2011).

5.3.3. Inositol-monophosphate and Glutathion S-tranferases

Inositol monophosphate is associated with lipid metabolism. Kocher et al. (2013) found seven members of this gene family in *L. albipes*. However, the expansion in this species probably reflects the life history of this species mainly when the foundresses are destined to survive through winter and undergo a diapause in their adult stage. After the diapause, foundresses have to find a new nest in the spring therefore sufficient nutrient storage is needed in order to survive the winter. *A. mellifera,* on the other hand has only three genes in this family.

Glutathion S-transferases are a group of multifunctional detoxication enzymes. Their main function is to catalyze the conjugation of reduced glutathion with elecrophilic substrates. Those conjugates are eliminated from the cell via the glutathione S-conjugate export pump (Simon 1996). This family also plays a role in intracelullar transport, hormone biosynthesis and protection against oxidative stress (Enayati et al. 2005). *Lasioglossum albipes* genome has an expanded Glutathion S-transferase family. *Lasioglossum albipes* contains nine members of this gene family, *A. mellifera* four members of this gene family. In *N. vitripennis*, 19 cytosolic glutathion S-transferases genes have been identified. Unlike in *A. mellifera*, where 2 microsomal glutathion S-transferases are present, *N.vitripennis* has only 1 microsomal glutathion S-transferase (Oakeshott et al. 2010). In *C. calcarata* there is also an expansion of glutathion S-transferase family namely OG5_127168 which is present in two copies (Rehan et al. 2016).

5.4. Micro RNA

MiRNA is a noncoding RNA acting as a translation repressing protein. MiRNA interracts with the 3´UTR of mRNA and its size is only 18-24 nucleotides (Søvik et al. 2015). In honey bee, the majority of miRNAs are located in intergenic regions. Nevertheless, a great deal can be also found within protein coding regions, primarily in introns (Ashby et al. 2016).

Shi et.al (2015) found a differential expression of miRNA in worker honey bee larvae and queen bee larvae. Some of the miRNAs were a target of up-regulation, some of them were equally expressed and other were down-regulated in queen bee larvae in comparison to the worker bee larvae, which confirms Ashby et al. (2016) and Greenberg et al. (2012). The up-regulated miRNAs *ame-let-7* is most likely related to the Dnmt1 (DNA methyl trasferase 1 (GB48403)), HDAC1(histone deacetylase 1 (GB53438)), p38b (Drosophila MAP kinase (GB43914)), Sirt6 (protein deacetylase (GB51490)) which are potential candidates for DNA methyltransferase activity, Na-dependent histon deacetylase activity, reactive oxygen species and the insulin signaling pathway. Some of these appear to be upmethylated (Dnmt1, p38) in a genome wide analysis of the honey bee (Shi et al. 2013). Also 37 miRNAs related to insulin pathway are differentially expressed in queen larvae and worker larvae and all of them were involved in caste differentiation. According to Ashby et al. (2016), GO and pathway analysis were used

to show queen larvae genes to be targeted by enrichment in pathways associated with physiometabolic processes and biosynthesis of juvenile hormone.

Bombus terrestris genome revealed a mirtron (miRNA located in intron) - bte-miR-6001 which contains the entire fourth intron of a Very High Density Lipoprotein (Vhdl), a gene with homology to Vitellogenin (Vg). The finding suggest that Vhdl is a candidate for a caste-associated gene in eusocial Hymenoptera in general according to Collins et al. (2017). *Apis mellifera* queens have a higher concentration of Vg mRNA in thorax when they are older, the same result applies to Vg mRNA concentration in heads (Corona et al. 2007). Also, Vitellogenin expression was higher in active queens (6 times) than in virgin queens or sterile workers (Shpigler et al. 2014). This mirtron is conserved in the same intron of the same gene in *A. mellifera*. Two miRNAs, Bte-miR-6001-5p and Bte-miR-6001-3p are more highly expressed in queen destined larvae in *B. terrestris*. This suggests the association of those miRNAs in queen-worker caste determination and/or differentiation in larvae stage (Collins et al. 2017). Simola et al. (2013) using RNA-seq gene expression data confirmed 115 miRNAs expressed in an ant *C. floridanus* where several of them are linked to caste specific and stage expression. MiRNAs and other regulatory sequences gather more in intergenic sequences. Some miRNAs target different castes and stages of life more often. Ant intergenic sequences are enriched for transcription factor binding sites or other regulatory elements.

MiRNA libraries containing a wasp *P. canadensis* and an ant *D. quadriceps* identified 159 miRNA families, 4 families unique to hymenopterans. There appear to be no miRNAs that were preferentially present in differentialy expressed genes (DEGs) specialising on phenotype specificity, some DEGs were highly targeted, others were not. As Patalano et al. (2015) stated, further work is needed to investigate miRNA expression levels to avert a role for miRNAs in caste differentiation in *D. quadriceps* and *P. canadensis*.

Nasonia vitripennis has the majority of miRNA sequences derived from *A. mellifera.* It is a reason of close proximity of those species in evolution (Sathyamurthy and Swamy 2010).

To evaluate the meaning of miRNA in the role of eusociality, examining primitively social species might help get us an insight into its value for the evolution of sociality. However no studies evalueting miRNA in primitively social species are available.

5.5. Differential expression

Queen and worker castes are produced from the same genome in most species therefore the genetic toolkit underlying convergent social caste phenotypes depends on the differential expression of common genes and/or pathways (Durant et al. 2016). A part of the differential expression problematics has already been presented in previous chapters (expanded/contracted gene families, miRNA, methylation). In *A. mellifera*, Insulin-like growth factor-1 and Juvenile hormone are involved in queen-worker caste determination on the molecular level (Smith et al. 2008). Developing queens show upregulation of

several of these genes unlike worker larvae, which show a low expression (Wheeler et al. 2006). Mutti et al. (2011) presented outcomes for Insuline like peptides levels for worker larvae. Insuline-like-peptide 1 expression was decreased, Insuline-like-peptide 2 was increased. Since Insuline-like-peptides (ILPs) considerably affect metabolism, nutrient storage, fertility and aging (Nilsen et al. 2011), looking for fluctuations of those ILPs might give us an insight into the basic pathways of caste determination in different species either primitively social or eusocial.

The fat body gives potential for ILP storage and expression. The fat body can be compared to the adipose tissue and liver in mammals. The nutrients which are present in fat bodies corelate with behaviour (Nilsen et al. 2011). Although Wyman and Richards (2003) did not succeed in finding evidence for fat bodies cooperating with the caste determination in *L. malachurum*, which is a primitively but obligatory eusocial species (Paxton et al. 2002).

The up-regulated transcripts of *C. calcarata* autumn mothers and dwarf eldest daughters were noticably enriched in GO enrichment levels for protein metabolic and aromatic compound biosynthetic processes. Spring and summer mothers, which belong to the reproductive caste, had differentially expressed transcript up-regulated in GO enrichment terms for reproduction, development and cell growth processes. In dwarf eldest daughters and autumn mothers, 180 transcripts which stand for 7% of all differentially expressed transcripts were only expressed in these two castes. Autumn mothers present a "postreproductive maternal care" and dwarf eldest daughters a "non-reproductive sibling care". One annotated transcript of interest is an Odorant Binding Protein 1 precursor (Rehan et al. 2014).

In socially polymorphic bee *H. rubicundus*, there were no significant differences between mean expected heterozygosity per locus between solitary and social populations, the difference in allele richness between the two phenotypes was also not significant. Also, the Bayesan clustering analyses targeted populations from the same place together and ignored the same phenotypes. This is an implication that geography is the main factor of determining genetic differentiation rather than the differences in expression of phenotypes (Soro et al. 2010). *Megalopta genalis* castes express differences in both brain and abdominal tissues. Abdominal tissue was targeted by gene expression, which seemed to be quite similar in workers and also in reproductives therefore differential castes are not significant in this case. Solitary females report high expression of genes similar to the queen's. Brain tissue gene expression revealed no significant differences among queens and workers, however, in solitary females and replacement queens differences were prominent. The interesting part is the high connection between bumblebee *B. terrestris* queens and sweat bee *M. genalis* queens, where 49% compliance corresponds in abdominal tissues and is more expressed in terms of GO enrichments (cell cycle (GO:0007049), metabolic proces (GO:0006259), nucleobase-containing compound metabolic process (GO:0006139)) (Jones et al. 2017). Jones et al. 2017 suggests that their outcomes might be a sign of common pathways across different types of eusociality where the species' origins are shared. This idea further teases Hunt et al. (2010), who also found a differential expression of 16 genes, where 12 of them are caste and/or diapause related. Some of them are expressed in nutrient metabolism, signaling pathways and caste

determination in eusocial species such as *A. mellifera* therefore some similarities might be considerable and might share similar pathways in different levels of sociality.

Caste differential expression in *P. dominula* exhibits enrichments for GO functions in neurotransmitter activity, amino acid metabolism and fatty acid metabolism (Standage et al. 2016), *P. canadensis* also show differentially expressed genes connected with caste differentiation, where workers show the intermediate levels of expression of genes associated with caste differentiation. The level of expression increases with other castes (81% of the genes were up- or down- regulated in those castes of queens and young females) (Sumner et al. 2006). Lipid metabolism, heat and stress response and solitary behaviour was a part of 389 differentially expressed genes in different castes in another paper wasp *P. metricus*, 45% of 389 differentially expressed genes were found to be influencing foraging/provisioning activity, 14% took part in reproduction and 38% cooperated with these two functions (Toth et al. 2010). Berens et al. (2014) found 736 differentially expressed transcripts in *P. metricus* castes of queen and worker, 91.7% of them were up-regulated in worker larvae than in queen larvae, 16 of which were related to heat and stress response and metabolism which is in agreement with Toth et al. (2010).

5.6. Gene regulation, Transcription factors

Kapheim et al. (2015) found out that the transition from solitary to group life is associated with an increased the capacity for gene regulation. Kapheim et al. (2015) searched for particular Transcription Factor Binding Sites (TFBSs) in the ten bee species using *D. melanogaster* Transcription factors (TFs). The connection between TFBSs and TFs was 89% succesfull. Transcription factors in terms with the results have increased capacity for gene regulation targetting eusocial species rather than solitary species. One-hundred and sixty-two genes out of 5,865 single-copy orhologs were targeted by accelerated evolution in species with more complex societies and were enriched in regulation of transcription, ribosomal structure, regulation of translation and RNA splicing. Woodard et al. (2011) also found bees of a different social background to have the same enrichments for the regulation of transcription (GO:0045449, GO:0006355), translation (GO:0006412), translation initiation (GO:0006413), RNA processing (GO:0001505), chromosome organisation (GO:0051276), chromatine remodeling (GO:0006338), chromatine modification (GO:0016568) and RNA splicing (GO:0000398, GO:0000377) as in Kapheim et al. (2015), in Roux et al. (2014) GO enriched genes connected to translation (GO:0006412), chromatine modification, organisation, remodeling, chromosome organisation and condensation, and translation.

Comparative study of seven ants (Simola et al. 2013) came up with the occurence of 11 significant expansions and 9 significant contractions, where 55% of them were expanded and possess DNA-binding capacity, 22% were contracted. Both groups may be involved in regulation of transcription. This according to Simola et al. (2013) suggests the importance of changes in the transcription factor repertoire for the initial stages of ant evolution. Ant genomes recognised 292 genes which are enriched for

categories involved in TF activity, intergenic sequences for regulatory elements, also miRNAs, non-coding RNAs and TFBSs already mentioned in this thesis.

In *A. mellifera*, the transcription factor Krüppel-homolog 1 (Kr-h1) was found to have downregulated expression in worker brains when queen pheromone or queen herself was present. In bumble bees, similarly to *A. mellifera*, comparing workers with active ovaries and inactive ovaries, the Kr-h1 is downregulated in workers with undeveloped – inactive ovaries. According to Shpigler et al. (2010), Kr-h1 transcription factor could transcriptionally regulate a conserved genetic element functioning in a pathway which participates in social behavior. Therefore it adjusts the behavior of workers to their socially environmental context.

Kapheim et al. (2015) also identified lineage-specific differences in coding sequences and promoter regions of 1,526 "social genes". Among these genes, common patterns of cis-regulatory evolution were found. Promoter regions gained transcription factor binding sites in the genes that evolve more slowly with increasing social complexity. In ants *A. echinatior* and *S. invicta* gene enrichment of Zinc ion binding was found (GO:0008270), the same was lost in *A. echinatior* but present in *S. invicta*, *C. floridanus* and *H. saltator*. Zinc finger IPR007087 was contracted in *A. echinatior*. In Kapheim et al. (2015), Zinc finger proteins function as transcription factors. An increased number of Zinc finger binding sites can be seen in eusocial versus solitary species.

Rehan et al. (2016) found an expansion of Zinc finger protein of C2H2 type (OG5_158738) in *C. calcarata* which was not found in any other bee genome. The Zinc finger (OG5_126627) was found to have 50 copies in the genome, in comparison to *A. mellifera* where there are two and *L. albipes* with the count of sixteen.

5.7. Positive selection

Positive selection is an action that promotes certain alleles which become prominent in the organism's phenotype (Vitti et al. 2013). Positive selection reflects the history of species and evolution therefore positive selection could be a factor which illuminates part of the mechanism of the evolution of eusociality.

In Roux et al. (2014) study of seven ant genomes, an enriched category for genes involved in immunity was detected and 1,832 single-copy orthologs were a target of positive selection which was common in the evolution of ants genes. Out of many enriched gene categories, immunity-related genes could help in disproving the hypothesis that hygienic behavior could weaken the selective pressure targeting immune genes in social insects. This, according to Roux et al. (2014) should be reflected in reduced levels of positive selection on these genes. This outcome is reflected in the honey bee genome (The Honeybee Genome Sequencing Consortium 2006) where fewer genes for innate immunity are present or in the evolution of innate immunity proteins in social insects (Harpur and Zayed 2013). These findings were previously mentioned by (Roux et al. 2014). Rehan et al. (2016) found genes that had undergone

positive selection in *C. calcarata*, when compared to the available ten bee species. Those genes were associated with lipid transport, protein binding and ribonuclease activity. In *C. calcarata*, those genes associated with DNA recombination are also evolving more quickly. Exceptionally high rates of meiotic recombination are found in social Hymenoptera, which was previously discussed (Wilfert et al. 2007). The social brain hypothesis presents that brains get substantially larger due to the demands of sociality in primates and some other mammals (Lihoreau et al. 2012). In insects, mushroom bodies are higher brain centres. Mushroom bodies are morphologically sophisticated complex enlarged structures in social insects (Farris and Schulmeister 2011) involved in learning ability and sensory integration (Ito et al. 1997). However, Roux et al. (2014) detected no signs of positive selection in genes involved in neurogenesis at the base of the Hymenoptera lineage. This supports *C. australensis*, that can be of two reproductive strategies, either solitary or social. No variation in the total brain volume was detected among reproductive strategies. Larger mushroom body calyx sizes were seen in dominant primaries. The least-developed mushroom body calyces were then noticed in solitary females acquiring no dominant or foraging statuses. This demonstrates according to Rehan et al. (2015), that sociality itself is not an explanation for mushroom body volume.

5.8. Neuropeptides

Neuropeptides are protein hormones which function as signaling molecules, neuromodulators in the peripheral and central nervous system and as regulatory hormones, which modulate central physiological processes like development, reproduction and behaviour, homeostasis, modulation of neuronal and muscular activity (Hauser et al. 2010; Nässel 2002). Neuropeptides are often coupled with G protein-coupled receptors. These receptors ensure the transduction of a signal which is transmissed by the neuropeptide (Brody and Cravchik 2000). Annotating neuropeptides is complicated since the product is first targeted by posttranscriptional processing in the form of a neuropeptide precursor therefore finding neuropeptides in the genome itself is quite complicated and takes a long time (Hummon et al. 2006). Hauser et al. (2010) found 20 core Neurohormone precursors in Arthropods (*Nasonia, Apis, Drosophila, Aedes, Bombyx, Tribolium, Acyrthosiphon pisum*) which Nygaard et al. (2011) subsequently used as a representative summation for his study of *A. echinatior* where the comparison of four ant species (*A. echinator*, *S. invicta*, *C. floridanus*, *H. saltator*) detected the same neuropeptide gene profile, which varies from other hymenopterans. Twenty-six neuropeptides, the superstructure for the 20 shared neuropeptides was the same for all four species used. The outcome, according to Nygaard et al. (2011), suggests a single common ancestor presenting a monophyletic clade. In *A. mellifera*, 36 neuropeptide genes were detected out of which 200 neuropeptides are produced (Hummon et al. 2006).

Two bumblebees, *B. terrestris* and *B. impatiens* genome consists of 34 neuropeptide precursors out of which 65 neuropeptides are produced. The set of neuropeptides for bumblebees is similar to that of *A. mellifera* with just two differences belonging only to one of each species, Trissin present only in bumblebees and Sulfakinin in *A. mellifera* (Sadd et al. 2015). Gospocic et al. (2017) found a neuropeptid Corazonin, which is downregulated by the workers of *H. saltator* that transform into gamergates (Figure 3). After injecting several individuals in the phase of dueling, which is caused by the decrease in corazonin titres, duelling ceased. Also, Corazonin encourages a hunting activity. Gospocic et al. (2017) performed a knockdown of a receptor for Corazonin and registered a cease in hunting activity. All of these attributes are a sign of gammergate transition. Gospocic says that higher levels of corazonin transcripts in workers of an ant *C. floridanus* were detected in Bonasio et al. (2010) and in a paper wasp *P. canadensis* in Patalano et al. (2015). In *C. calcarata*, the expansion of G-protein coupled receptor signalling pathway (GO:0007186) was a target of positive selection, also G-protein coupled receptor activity (GO:0004930, GO:0008528) was targeted (Rehan et al. 2016), however, no mention of Corazonin was found.

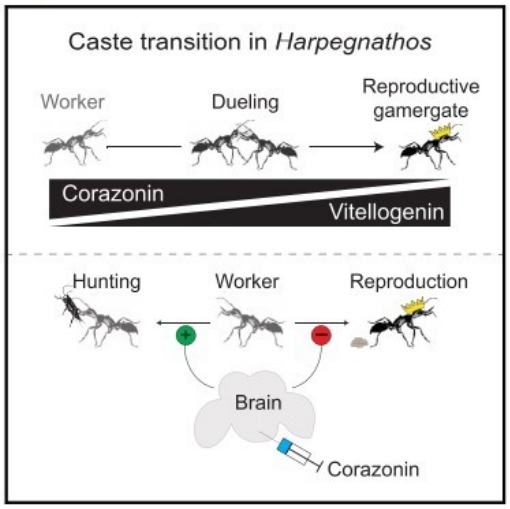

Figure 3. The influence of Corazonin on caste transition in Harpegnathos saltator. Vitellogenin biosynthesis increases with the decrease of Corazonin titres. The decrease of Corazonin also enhances agressivity and therefore the dueling behaviour between gammergates.

Injecting of Corazonin in the dueling worker causes hunting activity to emerge. Corazonin levels decrease in dueling workers not injected with Corazonin and activates the reproduction due to the Vitellogenin

6. Discussion and Conclusion

There are many studies concerning eusocial insects, not stating every study concerning eusocial species in the field of genomics, (The Honeybee Genome Sequencing Consortium 2006; Bonasio et al. 2010; Smith et al. 2011; Sirviö et al. 2011; Wurm et al. 2011; Nygaard et al. 2011; Gadau et al. 2012; Sirviö et al. 2006a), but there are far fewer publications concerning species from the simple stages of eusociality, insects such as *Ceratina calcarata* (Rehan et al. 2016) *Megalopta genalis* (Jones et al. 2015), facultatively eusocial *Lasioglossum albipes*(Kocher et al. 2013), primitively eusocial *Bombus terrestris* and *Bombus impatiens* (Sadd et al. 2015) or *Polistes dominula* (Standage et al. 2016). Also the studies of non eusocial but reflecting some kind of sociality are usually not comparative, therefore getting complex results is complicated. If more genomes of Hymenoptera with simple stages of eusociality or Hymenoptera with non eusocial lifestyles were available in the future, it would certainly improve our comprehending and approach towards analyzing data.

Many aspects undisputably contribute to eusociality and their identification is very complicated. We cannot say for sure what causes eusociality to arise in Hymenoptera, but similar patterns arrise in some aspects like recombination rate (Ross et al. 2015) or gene regulation (Kapheim et al. 2015; Rehan et al.

2016; Simola et al. 2013). Studies search for something the eusocial species have in common and compare those findings between each one of the eusocial and solitary species. For instance, castes have many independent origins in Hymenoptera. Berens et al. (2014) found out that the same genes are not always a part of the differentiation of castes among species that are not directly related, in this case honeybee *A. mellifera*, ant *S. invicta* and paper wasp *P. metricus*. Even paper wasps *P. canadensis* and *P. metricus*, which share the same divergence time and an ancestor, do not share the common novel differentially expressed transcripts involved in caste differentiation which might sound surprising. Looking for signs of the evolution of eusociality is therefore very complicated as we can see in this example, however even those outcomes offer us deeper knowledge about the species. A conserved set of genes or pathways causing eusociality to evolve through all eusocial Hymenoptera seems very unlikely but not impossible. For future research I suggest that continuing in comparative methods might give us important answers to many questions, however, focusing on individual species at the brink of eusociality and complete (as much as we are capable of) analysis of such species could help us understand the basis of sociality on a more complete level. Therefore working with such data could be helpful for the analyses in comparative studies and could suggest a right approach toward studying such phenomena. CRISPR Cas-9 appeared to be a very useful instrument in genome modifications, which gives us specific outcomes (Yan et al. 2017). Active usage of such instrument could help determining many unclear functions of genes at the genome. Preferential targetting of genes predicted to be a contribution to eusociality and should be the aim of this technique. More laboratory located ecological or molecular studies could help us see the differences. For example, Halictines are a great model for studying eusociality, this subfamily includes about 2400 species of which around 830 are eusocial (Schwarz et al. 2007). Halictini are socially polymorfic (Michener 2007) and laboratory induced conditions could shape solitary or social species and subsequent genomic data processing in different stages of transition could potentially reveal differences in genome structure or biosynthesis of components. However, those studies would be very hard to execute properly since genome sequencing and determining the function of new loci takes a lot of time and effort and also a lot of financial resources. Also, artificially applied components of hormonal system, signalling cascades, peptides and others could reveal plasticity in behaviour of an individual especially when such element applied is an element predicted to be a contribution to eusociality such as the neuropeptid Corazonin mentioned (Gospocic et al. 2017).

In my opinion, studies should be conducted from the bottom-top perspective. Focusing on solitary species might be the first step in understanding what enhances such behaviour. I suggest that searching for specific elements of the genome and the products is benefitial, but bringing more perspective and looking for elements that might not be obvious or apparent at the first sight is also very important and challenging for future studies. A very interesting discovery of the first biparental care in Apidae was made (Mikát 2014) and studying such species on the genomic level could be benefitial for understanding the emergence of eusociality thanks to the occurence of the eusocial behaviour in both, males and

females. Males in these species also contribute to the biparental care. Comparing males of *Ceratina nigrolabiata* with males of other species with uniparental female care might be as fruitful as comparing solitary species with eusocial species. Studying such phenomena in Apidae would also partially resolve the origin of biparental care in *C. nigrolabiata*. This study is connected to many ecological and behavioural features which should be a considerable part of every genomic and molecular research.

Likewise, we must not forget to look for result from the ecological point of view since it can partially elucidate the origin of many aspects such as chemoreceptors (Robertson a Wanner 2006) or fat bodies in species (Nilsen et al. 2011).

Also we must not forget that eusociality is connected not only to social Hymenoptera, but also to Termites (Thorne 1997), crustaceans (Duffy 1996; Duffy et al. 2002), mole rats (Jarvis 1981; Burland et al. 2002). Members of these groups should also be placed in comparative studies.

From what we know, methylation itself might not give answers strictly connected to eusociality because methylation patterns vary a lot among different taxa, where some of them are also not capable of de novo DNA methylation. Further research should be conducted with a connection to ecology and behaviour which could potentially explain the reason to what causes such a difference and whether methylation itself is connected to the evolution of eusociality in Hymenoptera or not. If there is a connection which would connect methylation to different types of eusociality the question is to what extent it influences such phenomena. MicroRNA is another mechanism which influences gene expression and is a promising mechanism in terms of evolution. Micro RNA is not a target of many recent studies but its potential to modify gene expression is auspicious. It could potentially be more prominent in the evolution of eusociality than methylation itself. What seems to be consistent are the results of the recombination rate for eusocial lineages of Hymenoptera. What stands behind the consistency is not perfectly explained yet. Contracted and expanded gene families of various types such as Yellow and Major Royal Jelly proteins or chemorecepors could be also connected to eusociality and its evolution, however, several publications contradict results which would confirm any hypothesis brought to elucidate the matter (Nilsen et al. 2011). We now cannot say how much and if it influences eusociality. Further important features of social interactions are neuropeptides. However, their detection is complicated (Hummon et al. 2006) and future studies are facing a great challenge. Substances similar to the neuropeptide Corazonin could be scattered all over the phylogenetic tree of eusocial Hymenoptera and therefore looking for such substances could bring a deeper understanding of the function of "social" neuropeptides and their function in eusociality. Whether substances similar to the neuropeptide Corazonin are present in other primitively social species is yet to be clarified.

Eusociality is still a complicated set of molecular, biochemical, ecological and evolutionary processes that is waiting for the great revelation. Nevertheless, we are now facing one of the greatest and most exciting challenges in the world of Hymenoptera - Decoding the Language of Eusociality.

7. Literature

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